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Relative Resistance to Chlorine of Poliovirus and Coxsackievirus Isolates from Environmental Sources and Drinking Water

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Several poliovirus and coxsackievirus isolates from environmental sources were compared with laboratory strains to determine their rate of inactivation by chlorine. All viruses were tested for up to 1,000 min in the presence of an initial free residual chlorine level of ca. 0.4 mg/liter. Coxsackievirus B5 (CB-5) isolates were found to be more resistant to chlorine than coxsackievirus B4 (CB-4), followed by poliovirus 1, 2, and 3 in order of decreasing resistance to chlorine. Environmental isolates of CB-5 were more resistant than the laboratory strain tested, and for two strains 12 and 22% of the input virus was still infectious after 100 min in the presence of free residual chlorine. Although CB-4 isolates were less resistant to chlorine than CB-5 isolates, after 1,000 min of contact 0.01% of the input virus was still infectious. Except for CB-5 isolates, isolates from environmental sources did not appear to be more resistant to chlorine than laboratory strains. Viruses isolated at different phases during the preparation of drinking water were not more resistant to chlorine and must thus have been protected by other mechanisms.

The selection of chlorine-resistant virus strains during water treatment has been postulated. However, the isolation of viruses from drinking water is rare, and these viruses are rarely tested for their resistance to disinfection. Shaffer et al. (11) have measured the resistance of two strains of poliovirus 1 isolated from drinking water and found these strains highly resistant to inactivation by chlorine at levels of up to 1.35 mg of free residual chlorine per liter. The individual resistance of enteric viruses can be quite different, and studies have shown striking differences between virus types (2, 7) and even between strains of the same serotype (11).

Our own laboratory has been involved in the evaluation of treatments at several water treatment plants and their ability to remove viruses and bacteria during the preparation of drinking water (9). Several isolates of enteroviruses were isolated from waters disinfected with chlorine. The present report analyzes the data obtained when these isolates and others isolated from sewage were tested to determine their relative resistance to chlorine disinfection when exposed to an initial concentration of ca. 0.4 mg of free residual chlorine per liter. The survival of tested strains was measured for up to 1,000 min to simulate water treatment conditions where the drinking water may remain in reservoirs or in the distribution system for ca. 24 h.

MATERIALS AND METHODS

Virus isolates. The virus isolates tested were obtained from various environmental sources, as well as from the Clinical Virology Laboratory at our institution (Institut Armand-Frappier). They are listed in Table 1 together with their sources. Poliovirus reference strains were obtained from the Quality Control Laboratory (Viral Vaccines) at our institute: type 1 Mahoney and Sabin (LSc-2ab), type 2 MEF-1 and Sabin (P712-CH-2ab), and type 3 Saukett and Sabin (Leon 12ab). All isolates were passaged once on BGM cells to obtain a sufficient amount of infectious supernatant for analysis.

Preparation of virus suspensions. Cell culture supernatants were centrifuged at 3,000 × *g* for 30 min and dialyzed overnight at 4°C against chlorine-demand-free water. The dialysate was centrifuged at 10,000 × *g* for 60 min, and the supernatant was filtered on a sterile 0.22-μm membrane filter. The suspensions were tested to evaluate their chlorine demand, and all were essentially chlorine demand free. Virus suspensions were stored at −70°C.

Preparation of chlorine-demand-free glassware and water. Chlorine-demand-free water was prepared from distilled water by the addition of sodium hypochlorite to obtain a free residual chlorine concentration of 3 mg/liter and maintained overnight. Residual chlorine was inactivated by placing the water in large beakers under UV light for 24 h. All glassware was immersed in distilled water containing 5 mg of free residual chlorine per liter overnight and rinsed several times in demand-free water.

Virus inactivation experiments. A stock solution containing 50 mg of sodium hypochlorite per liter was prepared from a commercial solution of sodium hypochlorite. One milliliter of the viral suspension to be tested was added to 200 ml of a 0.01 M calcium chloride solution at 5°C and pH 7.0 (±0.1) placed in a 500-ml hermetically closed glass bottle equipped with a stirring pad and two sampling ports (spinner flask). These bottles were siliconized to reduce virus adsorption to the glass. After mixing, a sample was taken to determine the initial virus titer. The addition of 2.5 ml of the stock solution of chlorine to the virus suspension allowed us to obtain a free chlorine residual of 0.4 to 0.5 mg/liter. Samples (5 ml) were taken at 1, 10, 100, and 1,000 min of contact at 5°C, while constant mixing was maintained at 80 rpm with a magnetic stirrer. No supplementary chlorine was added during the experiments. The final chlorine concentration after 100 min was 0.4 mg/liter, and after 16 h it was ca. 0.1 mg of free residual chlorine per liter and 0.4 mg of total residual chlorine per liter. The samples were immediately mixed with 5 ml of a 10-mg/liter sodium thiosulfate solution to inactivate the chlorine and were frozen at −20°C until assayed. All experiments were performed at least twice.

Chlorine determination. The free residual chlorine content of stock and treatment solutions was determined by am-

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TABLE 1. Virus survival after 1, 10, 100, and 1,000 min of contact with an initial concentration of 0.4 mg of free residual chlorine per liter

Virus	% Survival after min of contact:			
	1	10	100	1,000
Coxsackievirus B5 (no. 23, raw sewage)	83.33	70.00	21.67	0.079
Coxsackievirus B5 (no. 273, chlorinated water)	78.43	60.78	11.77	0.053
Coxsackievirus B5 (no. 241, chlorinated water)	44.76	3.43	0.24	0.041
Coxsackievirus B5 (laboratory strain)	19.82	1.44	1.22	<0.001
Coxsackievirus B4 (no. 1, treated sewage)	3.92	1.62	0.74	0.012
Coxsackievirus B4 (no. 358, chlorinated water)	4.38	0.31	0.063	0.014
Coxsackievirus B4 (no. 428, chlorinated water)	4.07	0.52	0.052	0.013
Coxsackievirus B4 (laboratory strain)	4.70	0.79	0.025	0.016
Coxsackievirus B4 (no. 469, chlorinated water)	4.13	0.35	0.023	0.011
Poliovirus 1 (no. 80, raw sewage)	10.49	0.90	0.029	0.014
Poliovirus 1 (Mahoney, laboratory strain)	8.95	0.72	0.029	<0.001
Poliovirus 1 (Sabin, laboratory strain)	1.17	0.023	0.004	<0.001
Poliovirus 1 (no. 4, raw sewage)	0.87	0.009	<0.001	<0.001
Poliovirus 2 (no. 426, chlorinated water)	0.96	0.10	0.033	<0.001
Poliovirus 2 (no. 533, chlorinated water)	1.33	0.092	0.020	0.002
Poliovirus 2 (MEF-1, laboratory strain)	1.23	0.090	0.010	<0.001
Poliovirus 2 (Sabin, laboratory strain)	0.26	0.035	0.006	<0.001
Poliovirus 2 (no. 454, chlorinated water)	1.13	0.021	0.003	<0.001
Poliovirus 2 (no. 7, raw sewage)	1.77	0.011	<0.001	<0.001
Poliovirus 2 (no. 42, raw sewage)	0.13	0.001	<0.001	<0.001
Poliovirus 3 (no. 25, raw sewage)	7.14	0.024	0.019	<0.003
Poliovirus 3 (Sabin, laboratory strain)	0.98	0.025	0.010	<0.003
Poliovirus 3 (Saukett, laboratory strain)	5.87	0.004	<0.003	<0.003
Poliovirus 3 (no. 51, raw sewage)	1.49	0.003	<0.003	<0.003
Poliovirus 3 (no. 26, raw sewage)	0.13	<0.003	<0.003	<0.003
Poliovirus 3 (no. 185, chlorinated water)	0.42	<0.003	<0.003	<0.003
Poliovirus 3 (no. 190, chlorinated water)	0.06	<0.003	<0.003	<0.003
Poliovirus 3 (no. 192, chlorinated water)	0.04	<0.003	<0.003	<0.003
Poliovirus 3 (no. 196, chlorinated water)	0.06	<0.003	<0.003	<0.003
Poliovirus 3 (no. 220, chlorinated water)	0.03	<0.003	<0.003	<0.003
Poliovirus 3 (no. 239, chlorinated water)	0.08	<0.003	<0.003	<0.003
Poliovirus 3 (no. 244, chlorinated water)	0.01	<0.003	<0.003	<0.003

perometric titration, whereas the residual chlorine of experimental solutions was determined by the diethyl-*p*-phenyldiamine colorimetric method (1).

Virus assays. Viruses were assayed in BGM cells by plaque formation under an agar overlay as previously described (9).

RESULTS

The relative resistance to chlorine of the virus isolates studied is presented in Table 1. Coxsackievirus isolates were the most resistant to chlorine inactivation, and two isolates of coxsackievirus B5 (no. 23, raw sewage, and no. 273, tap water) were relatively unaffected after 10 min of contact. After 100 min of contact, more than 10% of the virus was still infectious. After 1,000 min (ca. 16 h), more than 0.05% of the input virus remained infectious for these two isolates. The laboratory strain and isolate no. 241 (chlorinated filtered water from a filtration plant) were less resistant to chlorine than the two other field isolates, but were still more resistant than most other isolates, even after 1,000 min of contact. The laboratory isolate was completely inactivated after 1,000 min of contact, whereas the environmental isolate was still detectable.

Coxsackievirus B4 isolates were less resistant than the coxsackievirus B-5 isolates, but were more resistant than the poliovirus isolates. After 1,000 min of contact, ca. 0.01% of the virus was still infectious for all isolates, but differences in the inactivation rates were observed at 100 min of contact for isolate no. 1 (raw sewage). For this isolate 0.74% of the input virus was still infectious even if a rapid initial inactivation was observed, with only 3.92% remaining after 1 min but 1.62% remaining after 10 min.

Poliovirus isolates were less resistant to chlorine than the coxsackievirus isolates and were reduced to less than 0.003% after 1,000 min. A few isolates were slightly more resistant than the others during the first phase of inactivation (isolates no. 80 and no. 25, laboratory strains Mahoney and Saukett), but after a longer period of contact results were similar. Only strain no. 80 (poliovirus 1, isolated from sewage) appeared more resistant than the other isolates, with a survival rate equivalent to the one observed for the coxsackieviruses. Most poliovirus 3 isolates from water were easily inactivated by the free residual chlorine in less than 10 min, and the two laboratory strains of poliovirus 3 (Sabin and Saukett) were more resistant to chlorine than most of the water isolates.

DISCUSSION

The individual resistance of enteric viruses to chlorine has been revealed by the work of Liu et al. (7) in their classic study of 20 enteroviruses in river water. Since then, others have addressed the reasons for these differences, and factors like pH and temperature (2), the ionic environment (12), the aggregation state (5), and the conformational structure (8) of

viruses have been studied. These factors all contribute to the possibility that during drinking water treatment a more resistant strain of virus will survive water treatment practices considered adequate.

Viruses have been occasionally recovered from drinking waters considered safe by the generally accepted bacteriological and physicochemical standards (3, 4, 6, 9, 10, 13; P. Payment, R. Plante, and M. Trudel, submitted for publication). A recent report from England (13) has shown a high prevalence of viruses in drinking water samples: the yearly average was 16% of the drinking water samples tested positive for enteroviruses. The number of viruses in individual positive samples varied between 0.56 and 8.7 PFU/10 liters. Most of these viruses were isolated from water free of indicator bacteria and in the presence of residual chlorine. Our own results (9) from a 1-year study at each step of treatment at seven filtration plants has demonstrated the presence of a small number of viruses in ca. 7% of the finished water samples. The highest viral concentration recorded was 2 viruses per 100 liters, and the average concentration was 0.006 infectious units/100 liters. The surviving viral fraction after complete treatment was ca. 0.001% of the original indigenous virus population in the source water. This value is consistent with the data obtained in the present report on the chlorine resistance of these viral isolates.

Among the factors influencing viral resistance, aggregation and the presence of particulate or organic matter offer protection from chlorine disinfection. The fact that poliovirus isolates from drinking water or chlorine-treated waters are very sensitive to this disinfectant may indicate that they could have been protected from disinfection by aggregation or organic matter and not by increased resistance due to genetic modifications.

Several of the isolates tested in this study were isolated from water disinfected with chlorine, but they were not found to be more resistant than other strains of the same virus obtained from other sources. Coxsackievirus isolates from all sources, and particularly B5, were found to have a high degree of resistance to chlorine inactivation, and their presence in treated waters can probably be related to this higher resistance to disinfection. On the contrary, poliovirus isolates were generally found to be very susceptible to chlorine, and these viruses could have been protected from disinfection by the mechanisms mentioned earlier, particularly suspended solids.

Conclusions. Enteric viruses differ greatly in their resistance to inactivation by chlorine, but the results we have obtained show that some enteroviruses can survive for several hours in disinfected waters. Infectious viruses were still detected after 16 h in the presence of a residual chlorine concentration of ca. 0.1 mg/liter, a concentration equivalent to the one found in tap water in most distribution systems. This could explain why some viruses can survive water treatment practices involving chlorination and be detected in tap water. This residual fraction is, however, very small, and for most viruses less than 0.001% of the initial population will remain infectious after 16 h of treatment. No major differences could be demonstrated between isolates from waters treated or not treated with chlorine and laboratory isolates. The selection of resistant strains by water treatment practices thus appears improbable, but further studies are needed to evaluate a larger number of strains.

The very high resistance of some isolates of coxsackievirus B5, which after 100 min of contact with free residual chlorine was inactivated by less than 90%, does shed some doubt on the practice of chlorination without other treatments for the preparation of drinking water. It is also surprising that viruses were not detected more frequently in minimally treated drinking waters. However, the number of water samples analyzed for the presence of viruses is increasing every year, and methods have gained a high degree of sensitivity. It would thus be expected that the number of positive samples reported will also increase, as reported by Tyler (13) and ourselves (Payment et al., submitted for publication).

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